BMI and obesity risk also in Chinese, Japanese, Korean and Filipino populations [18–27]. A GWAS for BMI in 7,861 Koreans identified variation in *FTO* (rs9939609) as the most significantly associated locus, nearly reaching genome-wide significance ($p=1.5\times10^{-7}$) [28]. Furthermore, literature-based meta-analyses in Asians reported that the minor allele for the rs9939609 *FTO* single-nucleotide polymorphism (SNP) significantly ($p=9\times10^{-9}$) increased the risk of obesity, but no other obesity-related traits were examined [18, 29, 30]. Fewer studies in South Asians have been reported, two of which confirmed the association between the *FTO* locus and obesity susceptibility [31, 32], whereas one did not [33]. The prevalence of the risk allele in East Asians (~20%) and South Asians (~30%) is substantially lower than in Europeans, and the reported effect sizes in both East and South Asians vary widely for BMI (OR 0.13–0.83 kg/m^2 per minor allele) and obesity risk (OR 1.02–1.48 per minor allele) [16, 18, 20–25, 27, 34–39].

FTO was first identified as a type 2 diabetes-susceptibility gene, but, as further adjustment for BMI abolished the association with type 2 diabetes [1], it was suggested that *FTO* is primarily an obesity-susceptibility locus. However, the BMI-independent role of *FTO* in type 2 diabetes remains a matter of debate, particularly in Asians but also in white Europeans. While several studies have reported that the association between the *FTO* locus and risk of type 2 diabetes remained significant after adjustment for BMI [15, 18, 33, 35, 40, 41], others could not confirm this [21, 30, 32, 37, 42].

To firmly establish the association between the *FTO* locus and obesity susceptibility in East and South Asians and to assess its effect size and potential heterogeneity across Asian populations, we performed a systematic meta-analysis of data from 32 populations, including 96,551 men and women, using standardised study-specific association analyses. Furthermore, we examined whether the *FTO* locus is associated with type 2 diabetes independently of its association with BMI.

Methods

Literature search and study identification We designed a meta-analysis based on de novo analyses of data according to a standardised plan to achieve the greatest consistency possible across studies. We identified all published studies (before September 2010) that had examined the association of genetic variation in *FTO* with risk of obesity and type 2 diabetes and with obesity-related continuous traits in East and South Asian adults (age ≥18 years) by a PubMed literature search using the key words 'FTO', 'fat mass and obesity associated gene' and 'genome-wide association study'. References from the identified papers were subsequently screened to identify additional studies and to ensure that the list of eligible studies was complete. The literature search was carried out by two investigators independently, who cross-checked their search results for completeness.

Our literature search identified 38 publications, one of which was excluded because it was a subsample of another identified study. We invited the corresponding authors of the remaining 37 publications to join our meta-analysis, of which 26 agreed to participate and eventually 22 submitted raw data or summary statistics. We also included a Korean population with previously unpublished data (Y. M. Kim, J. Shin, C.B. Lee, M.K. Kim, Y. Tabara, T. Miki and B.Y. Choi), which was presented by a contributing author.

Taken together, our meta-analysis included data for 31 populations from 22 publications and one unpublished study, with 96,551 individuals altogether. The study identification and selection process is illustrated in Fig. 1.

All studies were conducted according to the Declaration of Helsinki. Informed consent was obtained from all participants, and the studies were approved by the ethics committees of the participating institutions.

Genotyping The rs9939609 *FTO* SNP was examined in 18 studies, whereas proxy SNPs were used in 14 studies. More specifically, the rs8050135 SNP was genotyped in 11 studies of East Asians and one of South Asians, and the rs3751812 and rs17817449 SNP were each genotyped once in studies of East Asians (electronic supplementary material [ESM] Table 1). The linkage disequilibrium between rs9939609 and the three proxies (rs8050135, 3751812, rs17817449) is perfect ($r^2=1$) in populations of East Asian origin, based on CHB+JPT data from the HapMap (Rel 24/Phase II). The linkage disequilibrium between rs9939609 and rs8050135 in Indian Asians is very high ($r^2>0.98$), based on a subsample ($n=305$) of the participating Lolipop study.

The genotyping success rate and concordance rate were >95%, and genotype distributions were in Hardy–Weinberg equilibrium ($p>0.01$) in all participating studies (ESM Table 1).

Statistical analysis As case–control definitions and statistical analyses used in the published papers were inconsistent, we asked analysts of each of the participating cohorts to re-analyse their data according to a standardised analysis plan. Summary statistics of each study were subsequently meta-analysed.

Obesity-susceptibility traits and type 2 diabetes Overweight was defined as a BMI ≥24 kg/m^2, and obesity as a BMI ≥28 kg/m^2 according to the definition proposed by the Working Group on Obesity in China [43]. Anthropometric data, including weight, height, waist circumference, hip

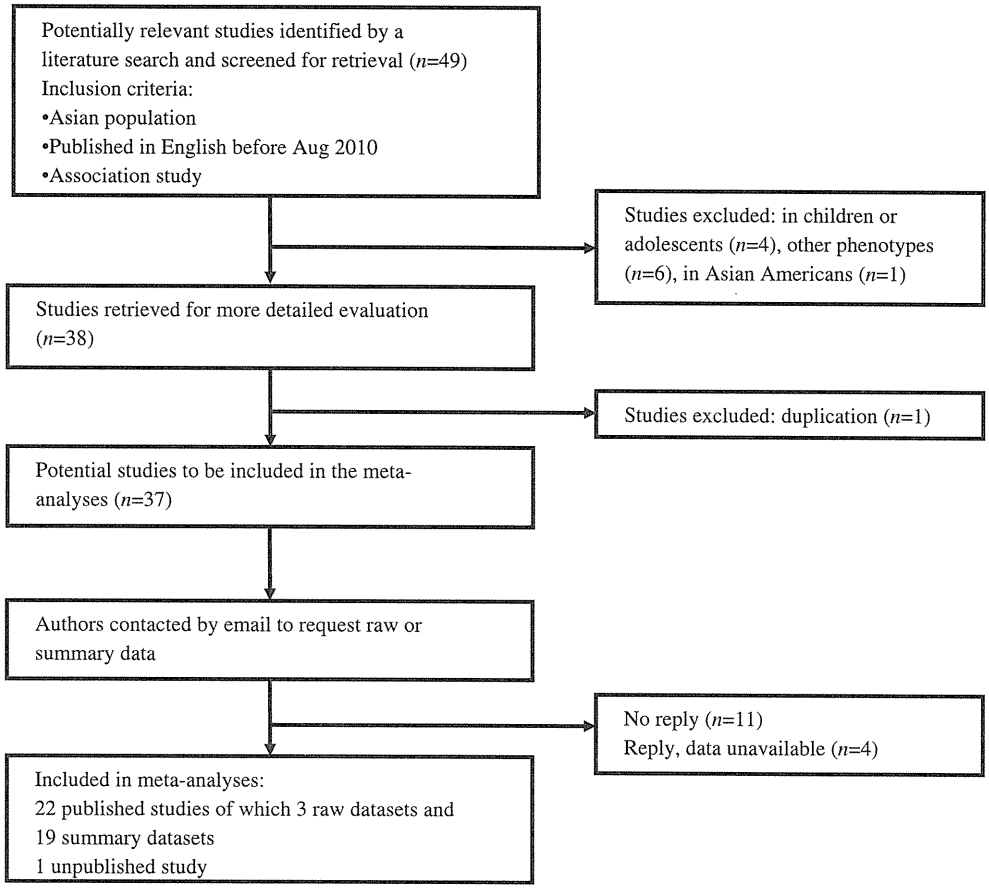

Fig. 1 Study identification and inclusion in the meta-analyses

circumference and body fat percentage, were collected in each study as described previously (ESM Table 1), BMI was calculated as weight (kg) divided by height squared (m^2), and WHR as waist circumference (cm) divided by hip circumference (cm). Raw data were used for analyses.

Type 2 diabetes was defined as meeting one or more of the following criteria: (1) fasting glucose ≥7.0 mmol/l; (2) 2-h glucose ≥11.1 mmol/l; (3) previous diagnosis of type 2 diabetes; (4) HbA_{1c} ≥6.5% (48 mmol/mol); (5) self-reported type 2 diabetes (ESM Table 1).

Study-specific de novo data analyses Association analyses within each study were performed for the total population and for men and women separately using additive and dominant genetic models. The associations of *FTO*-rs9939609 (or proxy) with risk of obesity and type 2 diabetes were assessed with multiple logistic regression models. Generalised linear models were used to assess the associations of *FTO*-rs9939609 (or proxy) with obesity-related continuous traits. In studies with a case–control design, analyses for continuous traits were conducted in control samples only. All analyses were adjusted for age and sex (sex-stratified analyses were only adjusted for age). The association with type 2 diabetes was also analysed with adjustment for BMI. Adjustments were performed by including the covariates (age, sex and/or BMI) as a linear term in the association model.

Summary statistics from the study-specific association analyses were reported in a standardised Excel form by the analysts of each study and collected centrally for meta-analyses.

Meta-analyses Data extraction from the forms and meta-analyses was performed independently by two investigators and cross-checked for consistency. All ambiguities were clarified with the respective analysts before the final meta-analyses.

ORs and beta coefficients from the individual studies were pooled using DerSimonian and Laird random-effects meta-analyses [44]. Meta-analyses were performed of all studies combined. Because of differences in genetic background as well as in susceptibility to obesity and type 2 diabetes, meta-analyses were also stratified by East Asian and South Asian origin of the populations. Furthermore, East Asians were further stratified according to their country of origin.

Between-study heterogeneity was tested by Cochrane's Q test and quantified by the I^2 index. I^2 values of <25%, 25–75% and >75% were defined as low, moderate and high heterogeneity, respectively [45]. To examine the sources of

heterogeneity in our meta-analyses, we performed random-effects meta-regressions, where the between-study variance was estimated with the restricted maximum likelihood approach. Meta-regressions included the following study-specific variables as covariates: year of publication, country of origin, sample size, study design, mean age and mean BMI.

A funnel plot, along with Begg's and Egger's tests, was used to test for the presence of publication bias.

Statistical analyses were performed with the Stata 9.0 software (StataCorp LP, College Station, TX, USA). Meta-analyses and meta-regressions were implemented by the *metan* and *metareg* commands of Stata, respectively. $p<0.05$ was considered to be significant, except for Cochrane's Q test for heterogeneity and Begg's and Egger's tests for publication bias, where a level of $p<0.10$ was used.

The variation in obesity-related continuous traits explained by the *FTO* variant was evaluated using the equation $2f(1-f)a^2$, where f is the frequency of the variant and a is its additive standardised effect [5]. Population-attributable risk (PAR) was calculated as $\mathrm{PAR} = (X-1)/X$. Assuming a multiplicative model, $X = (1-f)^2 + 2f(1-f)\gamma + f^2\gamma^2$, where γ is the estimated OR, and f is the frequency of risk allele [46].

Results

Characteristics of populations included in the meta-analyses Analyses were conducted in Chinese Hans (China Mainland: $n=10$; Singapore: $n=2$), Japanese ($n=7$), Indians ($n=7$), Koreans ($n=4$), Singapore Malays ($n=1$) and Filipinos ($n=1$; Table 1). Fifteen of the populations were case–control designed for obesity ($n=3$) or type 2 diabetes ($n=8$) or both ($n=4$), whereas 17 populations were population-based. The mean age and BMI of the populations ranged from 27.9 to 66.8 years and from 20.5 to 27.1 kg/m^2, respectively. The prevalence in population-based studies ranged from 3.1% to 37.9% for obesity and from 2.9% to 41.9% for type 2 diabetes.

The MAF of *FTO*-rs9939609 (or proxy) is 12–14% in Chinese Hans and Koreans, 18–20% in Japanese and Filipinos, and 30–33% in Singapore Malays and Indians (Table 1).

Associations with obesity and overweight A total of 24 populations ($n_{\mathrm{obese}}=13{,}032$; $n_{\mathrm{overweight}}=22{,}474$; $n_{\mathrm{normalweight}}=35{,}767$) were available for meta-analyses of the association between the *FTO* variant and risk of obesity and overweight.

Each additional *FTO*-rs9939609 minor (A) allele increased the odds of obesity by 1.25 ($p=9.0\times10^{-19}$) compared with normal weight individuals (Fig. 2), and by 1.17 ($p=7.4\times10^{-11}$) compared with non-obese individuals (ESM Fig. 1). Each additional minor allele increased the odds of overweight by 1.13 ($p=1.0\times10^{-11}$; ESM Fig. 2). The odds of obesity and overweight were the same in both East Asian and South Asian populations ($p=0.18$ and 0.84, respectively; ESM Table 2). Associations were similar in men and women (ESM Table 3). The heterogeneity across all studies was low ($13\%\leq I^2\leq19\%$).

When a dominant genetic model was used, the odds were only slightly higher than for the additive genetic model (ESM Table 4).

Association with type 2 diabetes In our meta-analysis of 22 populations ($n_{\mathrm{cases}}=33{,}744$, $n_{\mathrm{controls}}=43{,}549$), each additional *FTO*-rs9939609 minor allele increased the odds of type 2 diabetes by 1.15 ($p=5.5\times10^{-8}$) when adjusted for age and sex (Fig. 3). Further adjustment for BMI attenuated, but did not abolish, the association with type 2 diabetes (OR 1.10, $p=6.6\times10^{-5}$) (Fig. 4). Results were similar in East Asians and South Asians (ESM Table 2), in men and women (ESM Table 3), and when a dominant model was used (ESM Table 4).

The association results across all studies showed moderate heterogeneity ($44\%\leq I^2\leq48\%$; Figs 3 and 4). Meta-regression analyses revealed that the difference in study design contributed to some of the heterogeneity. Subsequent subgroup analyses showed that the association with type 2 diabetes was more pronounced in studies with a case–control design (OR [95% CI]=1.19 [1.14, 1.23], $p=3.7\times10^{-19}$, $I^2=0.0\%$) than in cohort studies (OR [95% CI]=1.09 [0.99, 1.20], $p=0.07$, $I^2=54.4\%$), which showed moderate heterogeneity (ESM Table 5).

Associations with obesity-related continuous traits The meta-analyses of the association of *FTO*-rs9939609 with BMI, waist circumference, hip circumference, WHR and body fat percentage included 30 ($n=71{,}022$), 22 ($n=51{,}543$), 20 ($n=48{,}508$), 20 ($n=48{,}508$) and nine ($n=19{,}580$) populations, respectively.

Each additional *FTO*-rs9939609 minor allele was associated with a 0.26 kg/m^2 higher BMI ($p=2.8\times10^{-17}$; equivalent to ~750 g/allele for a person 1.7 m tall) (Fig. 5), 0.51 cm larger waist circumference ($p=3.0\times10^{-9}$) (ESM Fig. 3), 0.36 cm larger hip circumference ($p=0.0003$) (ESM Fig. 4), 0.003 greater WHR ($p=1.2\times10^{-6}$; ESM Fig. 5), and 0.31% higher body fat percentage ($p=0.0005$) (ESM Fig. 6). All associations were very similar between East and South Asians (ESM Table 2), between men and women (ESM Table 3), or when a dominant genetic model was used (ESM Table 4).

We observed moderate heterogeneity across studies in the associations with BMI and hip circumference (BMI: $I^2=33\%$; hip circumference: $I^2=51\%$; Fig. 5; ESM Fig. 4). Meta-regression suggested that, for BMI, the heterogeneity was

Table 1 Descriptive information of studies included in the meta-analyses, sorted by ethnicity, study design and publication year

Paper	Study	Publication year	Ethnicity	Country	Study design	Sample size						Mean age (years)	Mean BMI (kg/m^2)	*FTO* SNP	MAF
						Obese	OW	NW	T2DM	NFG	QT analyses				
Li et al. [16]	NHAPC	2008	East Asian	China	Population based	472	1,215	1,503	423	1,893	3,190	58.62	24.43	rs9939609	0.11
Sha et al. [55]	GSBC	2009	East Asian	China	Population based	78	326	1,223	n.a.	n.a.	1,627	34.49	22.21	rs9939609	0.12
Hu et al. [56]	SHDS	2009	East Asian	China	Case–control[b]	n.a.	n.a.	n.a.	1,759	1,791	1,791	57.33	23.57	rs8050136	0.12
Li et al. [35]	WDS	2010	East Asian	China	Case–control[a, b]	243	976	1,368	877	1,405	1,405	44.23	21.45	rs9939609	0.12
Cheung et al. [24]	CRISPS	2010	East Asian	China	Case–control[a]	419	n.a.	691	n.a.	n.a.	691	44.98	21.19	rs8050136	0.12
Liu et al. [18]	n.a.	2010	East Asian	China	Case–control[a, b]	277	794	893	1,767	1,961	1,961	58.09	24.52	rs9939609	0.12
Ng et al. [21]	CUHK	2010	East Asian	China	Case–control[b, c]	1,147	2,293	2,432	5,872	583	583	41.31	22.87	rs3751812	0.12
Shu et al. [42]	SGWAS	2010	East Asian	China	Case–control[b]	n.a.	n.a.	n.a.	1,043	2,170	2,170	49.24	23.30	rs9939609	0.12
Wen et al. [57]	FLSGS	2010	East Asian	China	Case–control[b]	n.a.	n.a.	n.a.	1,160	1,127	1,127	59.09	24.13	rs8050136	0.12
Chang et al. [23]	NTUH	2008	East Asian	Taiwan	Case–control[a, b]	737	677	719	881	1,254	1,254	61.19	21.60	rs9939609	0.14
Cha et al. [25]	Kirin	2008	East Asian	Korea	Population based	252	304	361	n.a.	n.a.	917	27.91	26.39	rs17817449	0.14
Cha et al. [58]	KCMS	2009	East Asian	Korea	Population based	61	261	688	n.a.	n.a.	1,010	43.14	22.77	rs8050136	0.12
Kim et al. (unpublished data)	YangPyeung Cardiovascular Cohort Study		East Asian	Korea	Population based	339	995	1,092	194	2,061	2,426	57.60	24.49	rs9939609	0.12
Ng et al. [34]	Korea SNUH	2008	East Asian	Korea	Case–control[b]	n.a.	n.a.	n.a.	758	629	629	64.70	23.52	rs8050136	0.12
Takeuchi et al. [59]	CAGE-Amagasaki	2009	East Asian	Japan	Population based	388	1,562	3,719	n.a.	n.a.	5,660	48.86	22.99	rs9939609	0.19
Takeuchi et al. [59]	CAGE-Fukuoka	2009	East Asian	Japan	Population based	721	3,763	8,076	n.a.	n.a.	12,560	62.59	23.05	rs9939609	0.19
Takeuchi et al. [59]	CAGE-BMI	2009	East Asian	Japan	Population based	168	607	1,006	n.a.	n.a.	1,781	66.82	23.69	rs9939609	0.20
Karasawa et al. [19]	Takahata	2010	East Asian	Japan	Population based	220	886	1,533	215	2,306	2,639	63.04	23.48	rs9939609	0.20
Hotta et al. [20]	GWASJPN obesity	2008	East Asian	Japan	Case–control[a]	1,559	n.a.	1,541	n.a.	n.a.	1,541	47.52	21.21	rs9939609	0.18
Omori et al. [37]	RIKEN T2D	2008	East Asian	Japan	Case–control[b]	n.a.	n.a.	n.a.	4,584	2,262	2,262	44.84	22.86	rs8050136	0.20
Takeuchi et al. [59]	CAGE-T2DM	2009	East Asian	Japan	Case–control[b]	n.a.	n.a.	n.a.	6,781	7,307	n.a.	64.35	23.47	rs9939609	0.19
Marvelle et al. [27]	CLHNS	2008	East Asian	Philippines	Population based	321	560	836	155	1,463	1,717	48.51	24.31	rs9939609	0.18
Tan et al. [22]	SP2	2008	East Asian	Singapore (Chinese)	Population based	195	624	1,609	145	2,248	2,430	48.11	22.88	rs8050136	0.12
Tan et al. [22]	SiMES	2008	East Asian	Singapore (Malays)	Population based	848	826	846	787	1,248	2,520	59.04	26.38	rs8050136	0.30
Tan et al. [22]	SDCS	2008	East Asian	Singapore (Chinese)	Case–control[c]	426	809	757	n.a.	n.a.	n.a.	64.27	25.34	rs8050136	0.14
Chambers et al. [6]	LOLIPOP (IA317)	2008	South Asian	India	Population based	727	858	536	434	1,651	2,247	48.22	26.83	rs8050136	0.33
Chambers et al. [6]	LOLIPOP (IA610)	2008	South Asian	India	Population based	2,479	2,647	1,423	1,780	4,715	7,060	55.38	27.14	rs8050136	0.32
Tan et al. [22]	SINDI	2008	South Asian	India	Population	760	910	858	974	1,348	2,528	58.01	26.20	rs8050136	0.33

Table 1 (continued)

Paper	Study	Publication year	Ethnicity	Country	Study design	Sample size						Mean age (years)	Mean BMI (kg/m^2)	*FTO* SNP	MAF
						Obese	OW	NW	T2DM	NFG	QT analyses				
Yajnik et al. [33]	Parthenon	2009	South Asian	India	Population based	136	320	511	n.a.	n.a.	967	32.44	23.76	rs9939609	0.33
Yajnik et al. [33]	PMNS	2009	South Asian	India	Population based	59	271	1,546	50	1,681	1,876	32.71	20.83	rs9939609	0.31
Sanghera et al. [40]	Sikh Diabetes Study	2008	South Asian	India	Case–control[b]	n.a.	n.a.	n.a.	1,138	765	765	50.85	26.25	rs9939609	0.31
Yajnik et al. [33]	WELLGEN	2009	South Asian	India	Case–control[b]	n.a.	n.a.	n.a.	1,967	1,681	1,681	32.39	20.50	rs9939609	0.31

Individuals from CAGE-T2DM study were selected from other three CAGE population-based studies

[a] Obese case–control study

[b] T2DM case–control study

[c] Obese case–control study conducted in T2DM cases

n.a., data not available or not used in meta-analysis; NFG, normal fasting glucose; NW, normal weight; OW, overweight; QT, quantitative trait; T2DM, type 2 diabetes

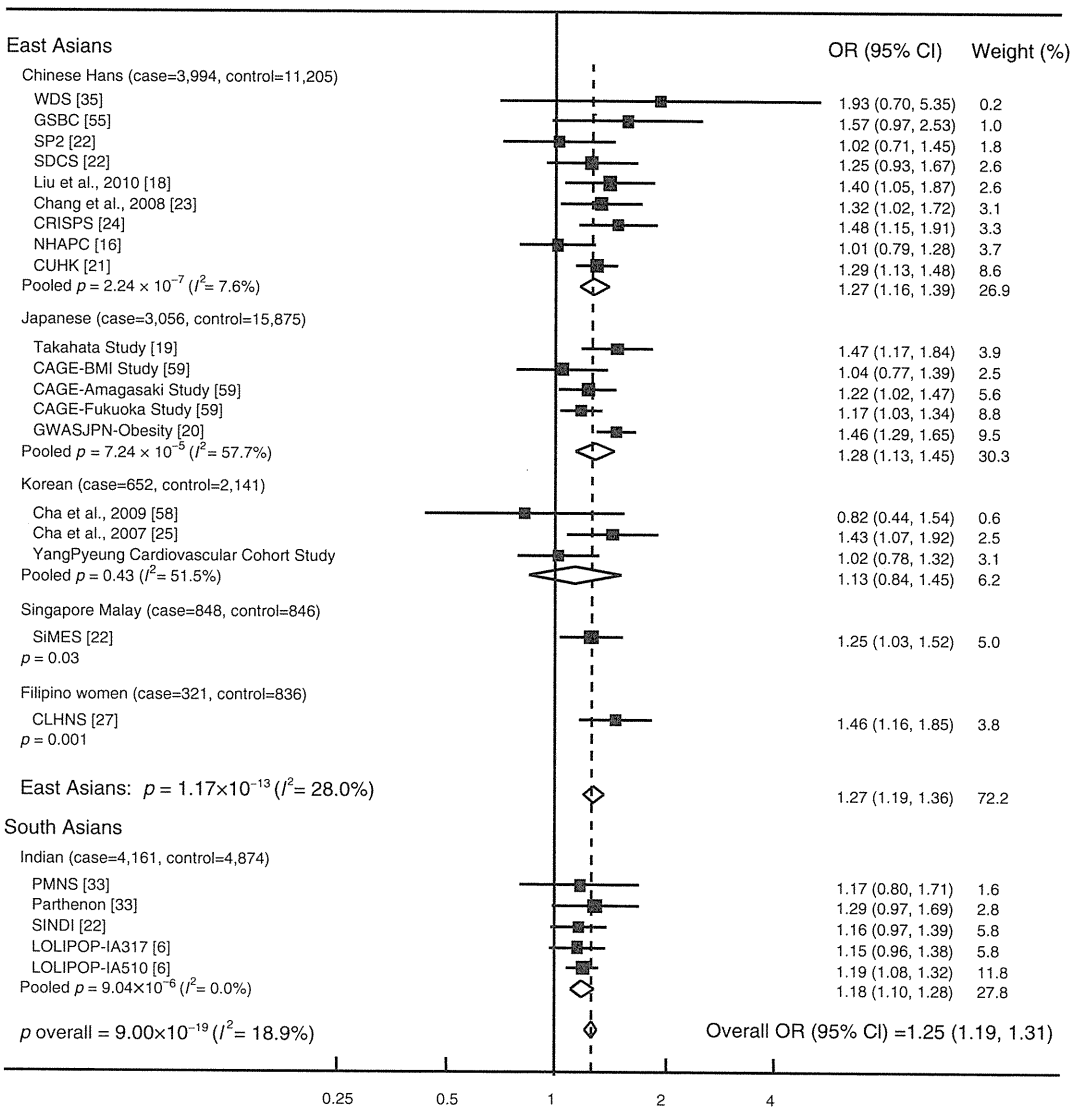

Fig. 2 Association of *FTO*-rs9939609 (or proxy) with obesity. Study-specific association analyses assumed an additive genetic model, comparing obese with normal-weight individuals, adjusted for age and sex. Effect sizes were combined using random-effects meta-analyses (DerSimonian–Laird method)

mainly due to difference in mean age and mean BMI among different populations. For hip circumference, the heterogeneity was mainly attributed to difference in mean BMI, i.e. the effect of the *FTO* minor allele tended to be larger in populations with a mean BMI ≥24 kg/m^2, compared with those with a mean BMI <24 kg/m^2.

FTO-rs9939609 explained 0.16% and 0.20% of the inter-individual variation in BMI in East and South Asian populations, respectively. The proportion of variation in other obesity-related continuous traits explained by *FTO*-rs9939609 was <0.10% (ESM Table 2).

Publication bias The funnel plots for the associations with obesity, type 2 diabetes, waist circumference, WHR and body fat percentage were symmetrical and the results for Begg's and Egger's tests were non-significant ($p \geq 0.10$), indicating that our results were not affected by publication bias (ESM Fig. 7). However, there was some evidence of publication bias and/or genetic heterogeneity for BMI (Begg's test, $p=0.08$; Egger's test, $p=0.07$) and hip circumference (Begg's test, $p=0.03$; Egger's test, $p=0.08$; ESM Fig. 7).

Discussion

This meta-analysis, combining data of 96,551 Asians from 32 populations, further confirms that genetic variation in *FTO* is associated with increased risk of obesity in East and South Asians. Despite differences in genetic background and obesity susceptibility between East and South Asians, the effect of *FTO* on obesity and related traits was generally

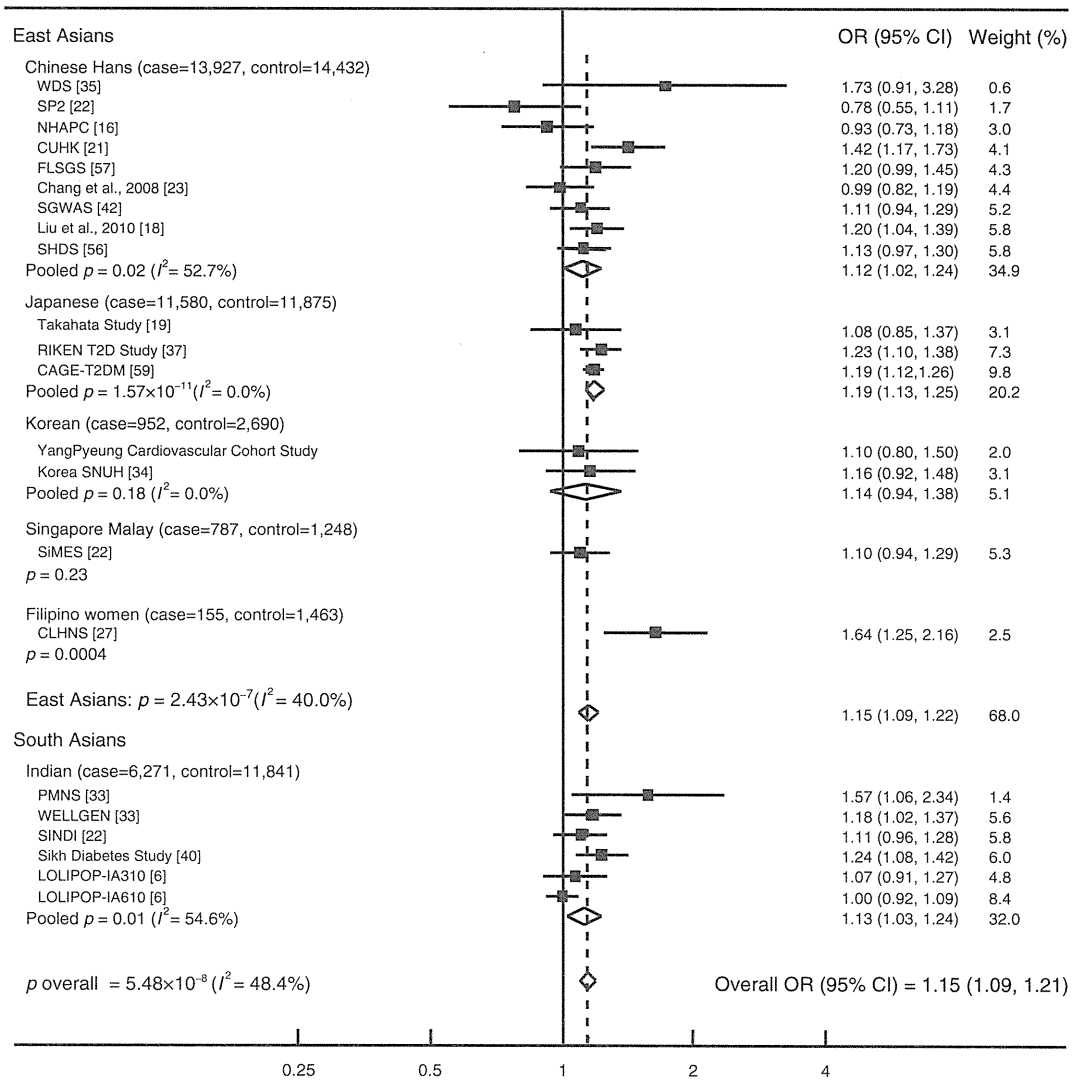

Fig. 3 Association of *FTO*-rs9939609 (or proxy) with type 2 diabetes. Study-specific association analyses assumed an additive genetic model adjusted for age and sex. Effect sizes were combined using random-effects meta-analyses (DerSimonian–Laird method)

similar to, or only somewhat smaller than, those reported for white Europeans. We furthermore confirm that variation in *FTO* is associated with increased risk of type 2 diabetes, an association that, unlike in white Europeans, is not abolished after adjustment for BMI in both East and South Asians.

Large-scale studies in individuals of white European descent have reported that each additional *FTO* minor allele increases the odds of obesity by 1.20–1.32-fold [1, 5, 7, 47]. The association with obesity observed in Asians in the present study was remarkably similar, with each additional minor allele increasing obesity risk by 1.25-fold (95% CI 1.19, 1.31), consistent with the association observed for obesity in previous literature-based meta-analyses of case–control studies in East and South Asians [18, 29, 30].

The association of the *FTO* variant with overweight was the same in East and South Asians (OR 1.13 per minor allele) and very similar to the effects (ORs ranging from 1.13 to 1.18) that have been reported in large-scale studies of white Europeans [1, 7, 47]. While the effect sizes observed for the influence of *FTO* on obesity and overweight in Asians are very similar to those of Europeans, it should be noted that the definitions of obesity and overweight are different, as BMI cut-offs are somewhat lower in Asians than in Europeans, consistent with the association of BMI with metabolic disease [48].

The *FTO* minor allele increases BMI by 0.26 kg/m^2 (equivalent to ~750 g/allele for a person 1.7 m tall) in Asians, with very similar results for East and South Asians. This observation suggests that the effect of *FTO* on BMI in Asians is substantially smaller than the effect observed in a meta-analysis of more than 125,000 white Europeans (0.39 kg/m^2 per minor allele, or 1,130 g per minor allele) [7]. This difference may be due to the fact that BMI in

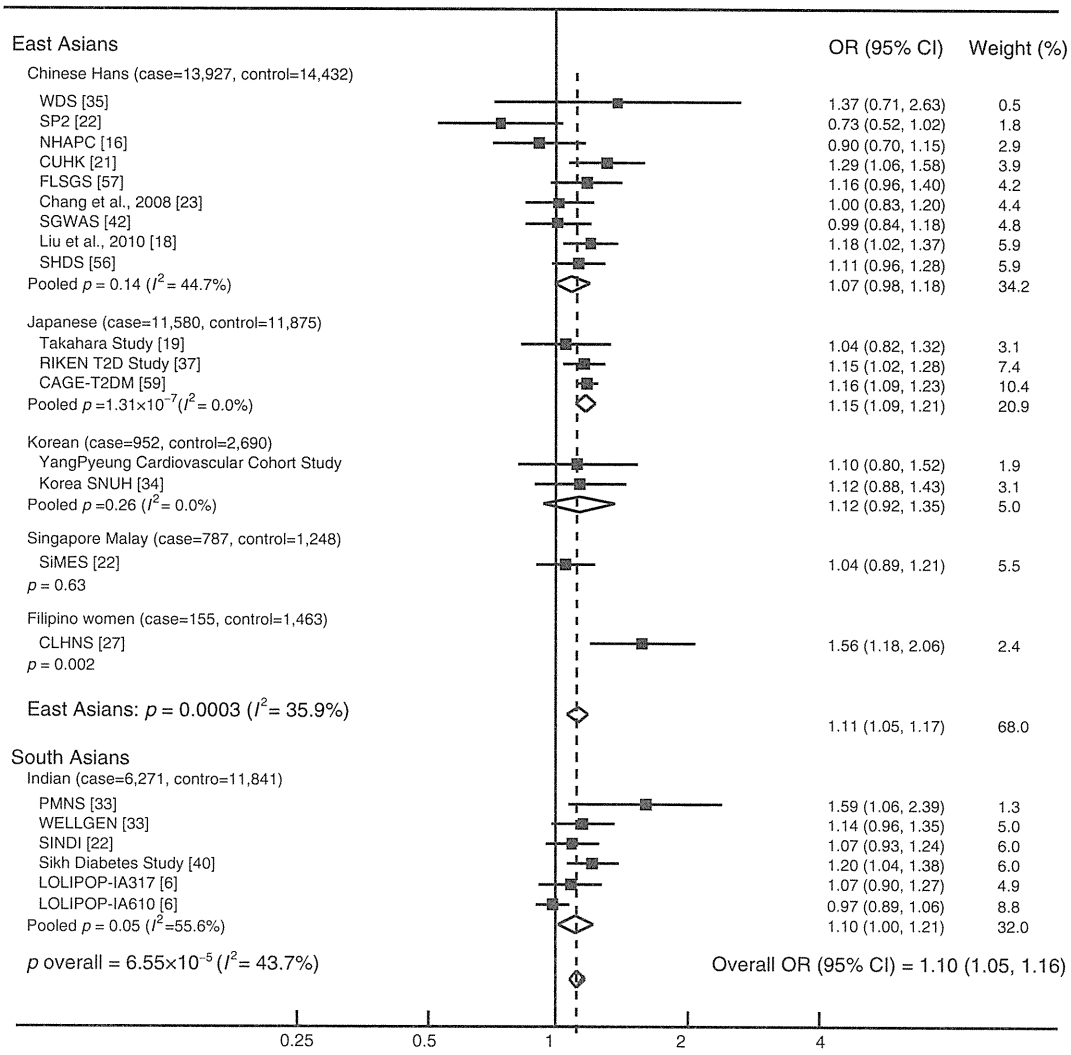

Fig. 4 Association of *FTO*-rs9939609 (or proxy) with type 2 diabetes adjusted for BMI. Study-specific association analyses assumed an additive genetic model adjusted for age, sex, and BMI. Effect sizes were combined using random-effects meta-analyses (DerSimonian–Laird method)

Asians does not represent exactly the same adiposity phenotype as in Europeans. However, given that other large-scale studies in white Europeans have reported effects for *FTO* on BMI that range between 0.26 and 0.39 kg/m^2, the comparison between Asians and Europeans should be made with caution [1, 3, 5, 15, 47]. The *FTO* variant also showed convincing association with measures of fat distribution such as waist and hip circumference and WHR in Asians. Despite the often described difference in abdominal obesity between East and South Asians, the effect sizes were very similar in the two groups. Consistent with the observations for BMI, the effect sizes tended to be somewhat smaller than those reported for white Europeans. For example, each additional *FTO* minor allele increased waist circumference by 0.51 cm in Asians, whereas large-scale studies in Europeans have reported an increase of 0.73–1.00 cm [1, 9, 47].

As the MAF of the *FTO* variant is substantially lower in Asians (East Asians, ~17%; South Asians, ~32%) than in white Europeans (~45%), and as the effect of this allele on obesity-related traits is similar or somewhat lower in Asians than in white Europeans, the overall contribution of genetic variation in *FTO* to obesity susceptibility will be lower in Asians, in particular East Asians. For example, the *FTO* variant explained less of the inter-individual variation in BMI in Asians (East Asians, 0.16%; South Asians, 0.20%) than in white Europeans (0.34%) [7]. Furthermore, the low risk allele frequency led to a lower PAR for the risk of obesity (East Asians, 8.3%; South Asians, 10.6%) and overweight (East Asians, 4.1%; South Asians, 7.8%) in Asians than in white Europeans (obesity, 20.4%; overweight, 12.7%) [1].

The *FTO* locus was first identified in a GWAS for type 2 diabetes in white Europeans, i.e. each minor allele

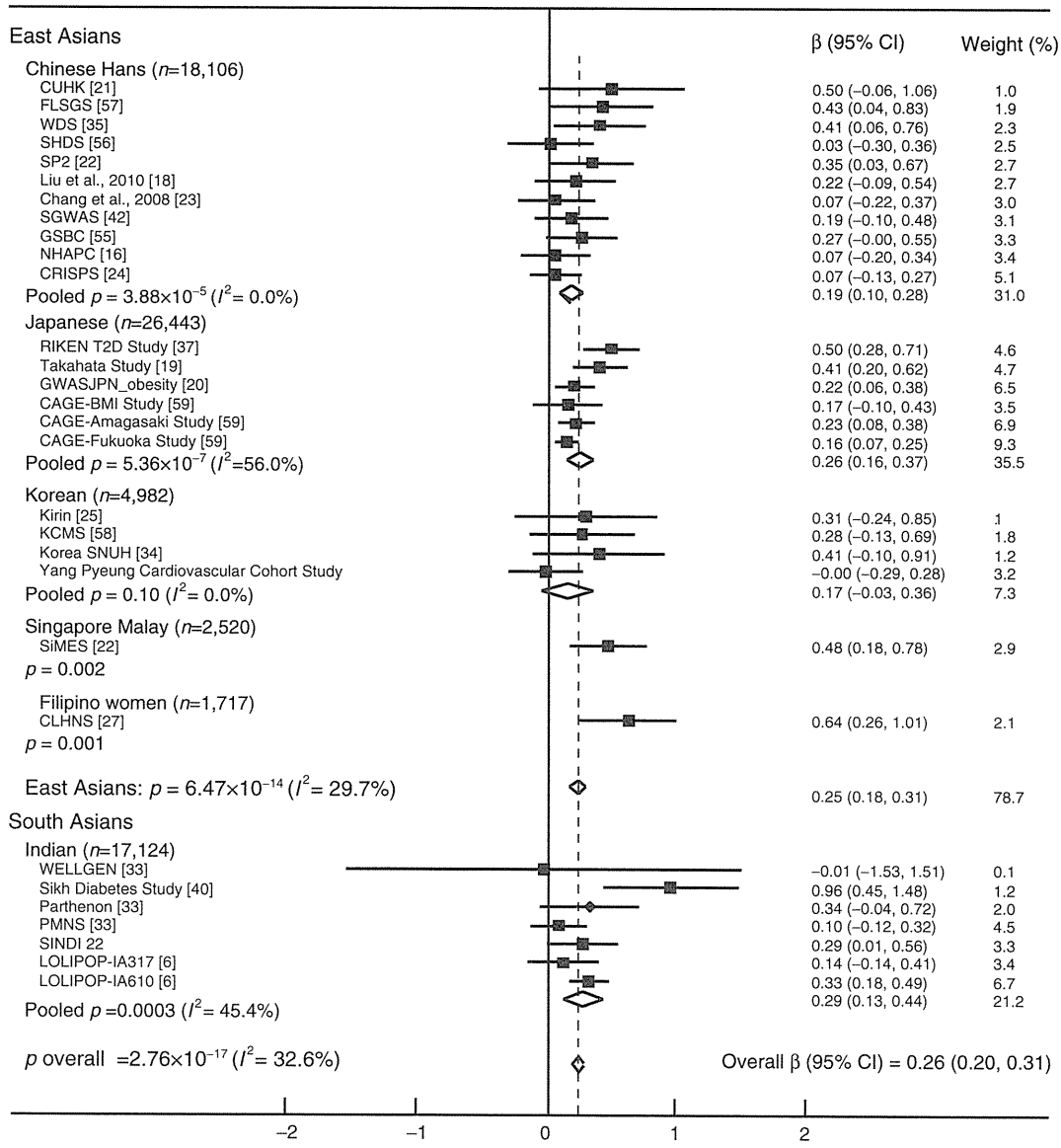

Fig. 5 Association of *FTO*-rs9939609 (or proxy) with BMI. Study-specific association analyses assumed an additive genetic model adjusted for age and sex. Effect sizes were combined using random-effect meta-analyses (DerSimonian–Laird method)

increased the odds of diabetes by 1.15-fold [1]. However, after adjustment for BMI, the association between the *FTO* variant and type 2 diabetes was completely abolished (OR 1.03), suggesting that *FTO* is primarily an obesity-susceptibility locus [1]. In our meta-analysis, we observed a similar effect of *FTO* on risk of type 2 diabetes, with each minor allele increasing the odds by 1.15-fold. Interestingly, adjustment for BMI did not abolish the association, but only slightly attenuated it to a 1.10-fold increased risk of type 2 diabetes for each additional minor allele. These observations were similar in East and South Asians, suggesting that the *FTO* locus influences the risk of type 2 diabetes, at least in part, independently of its effect on BMI. The reason for the discrepancy between the original observations in Europeans and our observations in Asians are not known, but may be due to the fact that *FTO* seems to have a smaller effect on BMI in Asians than in Europeans. It may also be due to the fact that BMI, as suggested above, represents a different adiposity phenotype in Asians than in Europeans because of differences in body composition. Although BMI is a marker for general adiposity, it does not distinguish between fat mass and fat-free mass and does not reflect regional fat distribution. Observational studies have suggested that, for a given amount of total body fat, East and South Asians have more abdominal fat and less muscle mass than white Europeans [49, 50]. However, while it has been generally believed that in white Europeans the association with type 2

diabetes is fully mediated by the effect of *FTO* on BMI, not all studies confirm this observation. A recent large-scale study in 41,504 Scandinavians found that the *FTO* minor allele indeed increased type 2 diabetes risk (OR 1.13), but this association remained present (OR 1.09) after adjustment for BMI, consistent with the observations in the present study. The biological pathways that underlie the independent association between *FTO* variation with obesity and type 2 diabetes remain unclear. However, results of gene expression studies have shown that *FTO* expression in human islets cells is not associated with BMI [51], whereas *FTO* mRNA and protein levels in muscle are increased in individuals with type 2 diabetes compared with non-diabetic obese individuals or healthy lean controls [52]. Furthermore, FTO overproduction in myotubes suggested a role for FTO in oxidative metabolism, lipogenesis and oxidative stress in muscle, a cluster of metabolic defects characteristic of type 2 diabetes [52].

Despite the fact that our meta-analyses included Asians with different genetic backgrounds, the overall heterogeneity of the association effects was generally only low to moderate. Interestingly, we found that the associations were generally very similar in East and South Asians, although these populations are known to have genetically different origins [53]. Furthermore, we found no differences between men and women, consistent with the observations in white Europeans [7]. We found some evidence that age may contribute to the heterogeneity of the association between *FTO* and BMI. Life course effects have been reported in white Europeans [54], and longitudinal analyses will be needed to establish this in Asian populations. Longitudinal studies are also more appropriate than cross-sectional studies for disentangling the intricate interplay between *FTO*, obesity and type 2 diabetes throughout life [15].

It should be noted that the association between *FTO* variation and obesity risk in Asians had been established in three earlier meta-analyses [18, 29, 30]. These meta-analyses were substantially smaller than the present ones and focused solely on case–control analyses of obesity and type 2 diabetes, while no continuous traits were studied. The meta-analysis by Liu et al [18] included individuals of East and South Asian origin, which were analysed together without comparison of effect sizes between the two populations. This study also examined the association with type 2 diabetes, but did not explore the association after adjustment for BMI [18]. Furthermore, the three previous meta-analyses were all literature-based and thus more prone to publication bias, whereas our meta-analysis was designed on the basis of a de novo analysis of data according to a standardised plan in all studies identified as having available data and agreement to participate. No evidence of publication bias was observed except for the associations with BMI and hip circumference. The analytical consistency across studies helped minimise between-study heterogeneity. Although our results are representative of individuals of Southeast Asian, East Asian and South Asian descent, the association of *FTO* with risk of obesity and type 2 diabetes in other Asian populations remains to be examined.

In summary, we have firmly established that genetic variation in the first intron of *FTO* is associated with increased risk of obesity and type 2 diabetes in Asians, with effect sizes similar to those in Europeans. Furthermore, we confirm that the association of *FTO* with risk of type 2 diabetes is partly independent of BMI.

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Contribution statement RJFL, HL and XL contributed to the conception and design of the study. CL and TOK performed the literature search, designed the analysis plan, performed the meta-analyses and researched the data. HL, TOK, CL and RJFL wrote the manuscript. HL, JZ, YL, CH, ZY, WZ, WB, SC, YW, TY, AS, BYC, CSY, DZ, FT, KY, JCC, KRM, LFB, MI, EN, NL, TF, SK, WW, CVJ, WL, YC, YX, YG, SL, YS, SHK, HDS, KSP, CHDF, JYK, PCS, KSLL, WZ, XS, HD, HI, GVK, DKS, LC, LL, RH, YK, MD, KH, WJ, JSK, JCC, GRC, RCM, SM, RD, MY, RT, NK, XL and RJFL collected study-specific data, analysed the study-specific data according to the standardised analysis plan, and reviewed and edited the manuscript. All authors have approved the final version of the manuscript to be published.

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nature genetics

Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians

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We conducted a three-stage genetic study to identify susceptibility loci for type 2 diabetes (T2D) in east Asian populations. We followed our stage 1 meta-analysis of eight T2D genome-wide association studies (6,952 cases with T2D and 11,865 controls) with a stage 2 *in silico* replication analysis (5,843 cases and 4,574 controls) and a stage 3 *de novo* replication analysis (12,284 cases and 13,172 controls). The combined analysis identified eight new T2D loci reaching genome-wide significance, which mapped in or near *GLIS3*, *PEPD*, *FITM2-R3HDML-HNF4A*, *KCNK16*, *MAEA*, *GCC1-PAX4*, *PSMD6* and *ZFAND3*. *GLIS3*, which is involved in pancreatic beta cell development and insulin gene expression[1,2], is known for its association with fasting glucose levels[3,4]. The evidence of an association with T2D for *PEPD*[5] and *HNF4A*[6,7] has been shown in previous studies. *KCNK16* may regulate glucose-dependent insulin secretion in the pancreas. These findings, derived from an east Asian population, provide new perspectives on the etiology of T2D.

T2D is a major public health problem with a rapidly rising global prevalence[8]. The development of T2D is influenced by diverse factors, and decades of epidemiological studies have linked obesity, hypertension and dyslipidemia with the risk of T2D[9]. It is also known that T2D has considerable heritability. Within only the last 3 years, genetic studies have produced a rapidly lengthening list of loci harboring disease-predisposing variations[10]. To date, genetic variants at 45 loci have been identified for T2D[10,11]. Despite these advances toward a better understanding of the genetic basis of T2D, its heritability has not been fully explained[12]. In addition, most of the T2D loci were detected initially in population samples of European origin, with the exceptions of *KCNQ1*, *UBE2E2* and *C2CD4A-C2CD4B*, which were first identified in studies of east Asian populations[13–15]. Additional efforts involving east Asian populations identified variants associated with T2D at the *SPRY2*, *PTPRD* and *SRR* loci[5,16,17]. However, these associations need more validation from additional studies of east Asians as well as in studies of other populations. A large meta-analysis in east Asians would be expected to identify new genetic associations and provide insights into T2D pathogenesis. In addition to differences in the allele frequencies between east Asians and Europeans, which may affect the power to detect associations in these populations, T2D epidemiology also differs considerably between European populations and east Asian populations. In east Asians, the rates of diabetes are often higher at lower average body mass indices (BMIs)[18], suggesting that some different pathways may be involved in pathogenesis of T2D in east Asians and Europeans.

To discover new T2D loci, we conducted a three-stage association study in individuals of east Asian descent (**Supplementary Fig. 1**). We performed the stage 1 meta-analysis by combining eight T2D genome-wide association studies (GWAS) participating in the Asian Genetic Epidemiology Network (AGEN) consortium (6,952 cases and 11,865 controls) with association data for 2,626,356 imputed and genotyped autosomal SNPs, and we used the inverse-variance method

A full list of affilations are at the end of article.

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for fixed effects for the statistical analyses (**Supplementary Table 1**). All imputed and genotyped SNPs (minor allele frequency (MAF) > 0.01) passed quality control filters in each of the eight stage 1 datasets prior to conducting the meta-analysis (**Supplementary Table 2**). The genomic control inflation factor (λ) for the meta-analysis was 1.046 (and was less than 1.062 for each of the individual studies), indicating that the results seen in stage 1 were probably not the result of population stratification (**Supplementary Fig. 2**). Individuals from each component study that participated in stage 1 mainly clustered together with the samples from the CHB/JPT HapMap population in the principal component analysis plot (**Supplementary Fig. 3**), further showing the similarity in ethnicity between the stage 1 samples. Most signals showing strong evidence for T2D associations were in known T2D genes (**Fig. 1**). Stage 1 *P* values, odds ratios (ORs) and average risk allele frequencies for 45 previously reported T2D-associated SNPs are listed in **Supplementary Table 3**.

After removing known T2D variants, we selected 297 SNPs from independent loci from the stage 1 meta-analysis based on our arbitrary inclusion criteria for *in silico* follow-up replication: meta-analysis $P < 5 \times 10^{-4}$ (based on the divergence between the observed and expected *P* values on the quantile-quantile plot; **Supplementary Fig. 2**), heterogeneity $P > 0.01$ and at least seven studies having been included in the meta-analysis (**Supplementary Table 4**). We took a total of 3,756 SNPs, including the 297 selected SNPs and their proxies ($r^2 > 0.8$ based on phase 2 CHB/JPT HapMap data), forward to stage 2 (*in silico* replication) in three independent GWAS (5,843 cases and 4,574 controls). After a meta-analysis that combined stage 1 and 2 data for 3,756 SNPs, we selected the 19 SNPs that showed the most compelling evidence for association (stage 1 and 2 combined $P < 10^{-5}$) (**Supplementary Table 5**) for stage 3 *de novo* genotyping in up to 12,284 cases and 13,172 controls recruited from five independent studies (**Supplementary Tables 1** and **2**). This resulted in eight new T2D loci that reached genome-wide significance in the combined meta-analysis across all three stages (**Table 1** and **Fig. 2**).

Three of these T2D-associated loci were previously associated with metabolic traits or related diseases or were suggestively associated with T2D. We detected one such locus within an intron of *GLIS3*, a gene that is highly expressed in islet beta cells. The coding product of this gene, a Krüppel-like zinc finger transcription factor, has been proposed as a key player in the regulation of pancreatic beta cell development and insulin gene expression[1,2]. SNPs in high linkage disequilibrium (LD) with this locus have been implicated in association with type 1 diabetes (T1D)[19] and fasting plasma glucose[3]. The second such locus, on 19q13, is located in an intron of *PEPD*. Several SNPs (lead SNP: rs10425678) in this gene were previously associated with T2D in a Japanese population[5]. However, the SNP in *PEPD* identified in our study (rs3786897) is not in LD with those identified in the Japanese population ($r^2 = 0.008$, $D' = 0.143$ between rs3786897 and rs10425678 based on phase 2 CHB/JPT HapMap data), and our GWAS data do not support an association for T2D with rs10425678 ($P = 0.528$). The third such signal is near *FITM2-R3HDML-HNF4A*. *FITM2* may be involved in lipid droplet accumulation[20], and the function of *R3HDML* is not known. Mutations in *HNF4A* cause maturity onset diabetes of the young type 1 (ref. 21). Common variants in the P2 promoter region of this gene (rs1884613 and rs2144908) have been associated with T2D in a population-specific manner[6,22]. The SNP near *FITM2-R3HDML-HNF4A* identified in our study (rs6017317) is not in strong LD with the *HNF4A* P2 promoter SNPs ($r^2 = 0.23$–0.25, $D' = 0.50$–0.54 between rs6017317 and rs1884613 or rs2144908 based on phase 2 CHB/JPT HapMap data), indicating that rs6017317 is a new T2D signal in the 20q13.12 region where *HNF4A* resides.

The other five loci reaching genome-wide significance in our study have not previously been reported in the context of any metabolic traits, including the loci mapped in or near *KCNK16*, *MAEA*, *GCC1-PAX4*, *PSMD6* and *ZFAND3*. *KCNK16*, which is expressed predominantly in the pancreas, encodes a potassium channel protein containing two pore-forming P domains[23]. In pancreatic β cells, potassium channels that are inhibited by ATP regulate glucose-dependent insulin secretion. Among the variants in strong LD with the signal reaching genome-wide significance in *KCNK16* (rs1535500) is rs11756091 ($r^2 = 0.977$, $D' = 1.0$ based on phase 2 CHB/JPT HapMap data), which encodes a substitution of proline to histidine in two isoforms of KCNK16. This variant or others influencing *KCNK16* may result in the defective regulation of potassium channel activity that contributes to the etiology of T2D[24]. *MAEA* encodes a protein that has a role in erythroblast enucleation and in the development of mature macrophages[25]. A gene-set analysis of the stage 1 *P* values using GSA-SNP[26] indicated that *MAEA* belongs to a group of genes that previously showed significant association with T2D and includes *IDE*, which is located at a known T2D susceptibility locus[27] (stage 1 $P = 1.41 \times 10^{-7}$ for rs6583826 at the *IDE* locus in this study). The GRIP-domain–containing protein that is encoded by *GCC1* might have a role in the organization of the trans-Golgi network, which is involved in membrane transport[28]. *PAX4*, which is only 30 kb away from *GCC1*, is a good candidate for T2D given its involvement in pancreatic islet development. *PAX4* was recently implicated in a Japanese individual with maturity onset diabetes of the young[29]. The expression product of *PSMD6*, which acts as a regulatory subunit of the 26S proteasome, is probably involved in the ATP-dependent degradation of ubiquitinated proteins[30]. Although the function of *ZFAND3* has not been fully elucidated, it is noteworthy that a member of the same gene family, *ZFAND6*, is present along with *FAH* at a previously detected T2D locus[31]. We examined whether eight new loci are potentially associated with T2D through an effect on obesity, as is the case with *FTO*[32]. All of the T2D association signals we initially detected remained after adjustment for BMI (**Supplementary Table 6**), indicating that the associations with T2D of these eight loci are not mediated through an effect on obesity.

In addition to the eight loci reaching genome-wide significance, we identified two loci showing moderate evidence (combined $P < 10^{-6}$) of association with T2D, including *WWOX* and *CMIP* loci (**Table 1**). We obtained the association results for these ten loci in GWAS data from up to 47,117 European samples generated by the DIAGRAM consortium (DIAGRAM+ is the current version of dataset)[31].

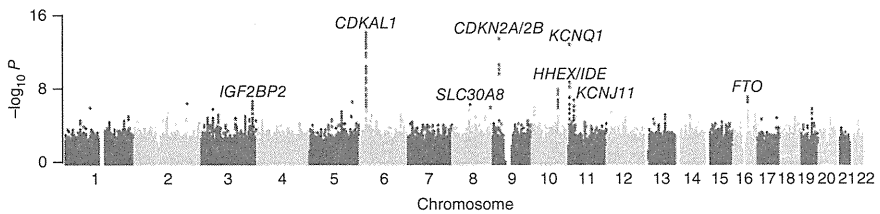

Figure 1 Genome-wide Manhattan plot for the east Asian T2D stage 1 meta-analysis. Shown are the $-\log_{10} P$ values using the trend test for SNPs distributed across the entire autosomal genome. The red dots at each locus indicate the signals with $P < 10^{-6}$ detected in the genome-wide meta-analysis. A total of 1,934,619 SNPs that were present in at least five stage 1 studies were used to generate the plot.

Table 1 Eight new T2D loci reaching genome-wide significance from a combined meta-analysis of stages 1, 2 and 3

SNP	Chr.	Position (bp)	Nearby gene	Risk allele	Other allele	Stage 1 (discovery)[a]		Stage 2 (*in silico* replication)[b]		Stage 3 (*de novo* replication)[c]		Combined (stages 1, 2 and 3)[d]	
						OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Loci showing strong evidence of association with T2D													
rs6815464	4	1,299,901	*MAEA*	C	G	1.09 (1.04–1.14)	8.21×10^{-4}	1.13 (1.07–1.20)	3.67×10^{-5}	1.16 (1.11–1.20)	4.15×10^{-15}	1.13 (1.10–1.16)	1.57×10^{-20}
rs7041847	9	4,277,466	*GLIS3*	A	G	1.09 (1.04–1.14)	1.29×10^{-4}	1.09 (1.03–1.15)	2.20×10^{-3}	1.11 (1.07–1.15)	2.89×10^{-9}	1.10 (1.07–1.13)	1.99×10^{-14}
rs6017317	20	42,380,380	*FITM2-R3HDML-HNF4A*	G	T	1.10 (1.05–1.15)	2.43×10^{-5}	1.07 (0.99–1.15)	8.42×10^{-2}	1.10 (1.06–1.14)	3.96×10^{-7}	1.09 (1.07–1.12)	1.12×10^{-11}
rs6467136	7	126,952,194	*GCC1-PAX4*	G	A	1.12 (1.06–1.18)	6.47×10^{-5}	1.11 (1.04–1.18)	2.09×10^{-3}	1.10 (1.05–1.15)	2.31×10^{-5}	1.11 (1.07–1.14)	4.96×10^{-11}
rs831571	3	64,023,337	*PSMD6*	C	T	1.11 (1.06–1.17)	4.85×10^{-6}	1.06 (1.00–1.13)	4.46×10^{-2}	1.08 (1.05–1.12)	1.41×10^{-5}	1.09 (1.06–1.12)	8.41×10^{-11}
rs9470794	6	38,214,822	*ZFAND3*	C	T	1.11 (1.05–1.17)	1.45×10^{-4}	1.09 (1.02–1.17)	1.48×10^{-2}	1.16 (1.09–1.23)	3.20×10^{-6}	1.12 (1.08–1.16)	2.06×10^{-10}
rs3786897	19	38,584,848	*PEPD*	A	G	1.14 (1.08–1.20)	3.74×10^{-6}	1.05 (0.99–1.12)	1.28×10^{-1}	1.11 (1.04–1.17)	5.46×10^{-4}	1.10 (1.07–1.14)	1.30×10^{-8}
rs1535500	6	39,392,028	*KCNK16*	T	G	1.11 (1.06–1.16)	5.34×10^{-6}	1.07 (1.01–1.15)	3.33×10^{-2}	1.06 (1.02–1.10)	3.50×10^{-3}	1.08 (1.05–1.11)	2.30×10^{-8}
Loci showing moderate evidence of association with T2D													
rs16955379[e]	16	80,046,874	*CMIP*	C	T	1.13 (1.07–1.20)	2.20×10^{-5}	1.10 (1.03–1.17)	6.59×10^{-3}	1.05 (1.01–1.10)	2.19×10^{-2}	1.08 (1.05–1.12)	2.84×10^{-7}
rs17797882	16	77,964,419	*WWOX*	T	C	1.12 (1.05–1.18)	1.76×10^{-4}	1.09 (1.02–1.16)	1.21×10^{-2}	1.06 (1.01–1.11)	1.61×10^{-2}	1.08 (1.05–1.12)	9.49×10^{-7}

[a]Up to 6,952 cases and 11,865 controls. [b]Up to 5,843 cases and 4,574 controls. [c]Up to 12,284 cases and 13,172 controls. [d]Up to 25,079 cases and 29,611 controls. [e]The proxy SNP rs9930117 ($r^2 = 1$) was genotyped in the stage 3 CAGE study.

The DIAGRAM-generated results for these loci indicated that three loci, including the *FITM2-R3HDML-HNF4A* (rs6017317: $P = 1.47 \times 10^{-2}$, OR = 1.07), *CMIP* (rs16955379: $P = 3.33 \times 10^{-2}$, OR = 1.20) and *MAEA* (using the proxy SNP for rs6815464, rs11247991 ($r^2 = 0.96$): $P = 6.56 \times 10^{-3}$, OR = 1.19) loci, were modestly associated with T2D, whereas a locus in *GLIS3* (rs7041847: $P = 6.43 \times 10^{-2}$, OR = 1.04) was nominally associated with T2D. The direction of effect was consistent in four (*PSMD6*, *PEPD*, *WWOX* and *KCNK16*) of the six loci that were not replicated in DIAGRAM+ (**Supplementary Table 5**).

We analyzed the functional connections among the 10 new T2D genes and the 28 known T2D genes that we replicated in this study (**Supplementary Table 3**) using GRAIL[33]. The connection results highlighted notable biological functions for sets of genes within T2D-associated regions (**Supplementary Fig. 4** and **Supplementary Tables 7** and **8**). For example, *KCNK16* has strong connections with previously known T2D genes encoding potassium channels (*KCNJ11* and *KCNQ1*), implying that is has a physiological role in the regulation of potassium transport in pancreatic cells.

We examined the association between each new T2D SNP and the expression of genes within 1 Mb of these SNPs by an expression quantitative trait locus (eQTL) analysis using the data from the MuTHER consortium. One SNP (rs3786897) in an intron of *PEPD* was highly associated with the mRNA expression of *PEPD* in the adipose tissue of 776 individuals of European ancestry ($P_{eQTL} = 2.14 \times 10^{-8}$) (**Supplementary Table 9**). However, this SNP did not show an association with T2D in populations of European ancestry, thus the importance of this finding is unclear.

We considered the possibility that autoimmune diabetes (rather than T2D) may be driving some of the signals that we observed. First, the cases from all the studies we examined predominantly had adult-onset diabetes (age of disease onset ≥30 years), and none of the clinically diagnosed subjects had T1D, which is defined by the presence of acute ketosis and the continuous requirement of insulin beginning within 1 year after diagnosis. Second, we researched the associations for all known T1D-associated variants in our dataset. Only a small number of loci showed association after this analysis (**Supplementary Table 10**). These results are in distinct contrast to those for known T2D-associated variants, many of which replicated in our study (**Supplementary Table 3**), further suggesting that our findings are most relevant to T2D. Third, as variants close to the *GLIS3* locus have been shown to be associated with T1D[19], we examined the association between rs7041847 and diabetes in four studies ($n = 8,383$) in which individulas with positive glutamic acid decarboxylase (GAD) antibodies had been excluded (as individuals with T1D are positive for GAD antibodies, whereas individuals with T2D are not) (data not shown). In each study, the associations between this SNP and diabetes were the same as the association found when we included all the samples (meta-analysis $P = 3.4 \times 10^{-4}$, OR = 1.12). This finding, along with the fact that SNPs at the *GLIS3* locus also show associations with fasting plasma glucose in nondiabetic adults[3] and in healthy children and adolescents[4], is consistent with the hypothesis that SNPs at this locus may affect fasting glucose homeostasis rather than the immune system. Taken together, it is unlikely that a substantial proportion of the positive associations observed in our study were driven by autoimmune diabetes.

This study is the largest GWAS meta-analysis, to our knowledge, conducted for T2D in east Asians. Findings from this study highlight not only previously unknown biological pathways but also population-specific loci for T2D. The association of rs9470794 in *ZFAND3* with T2D seems to be highly specific to east Asian populations (**Supplementary Table 5**), whereas the association of

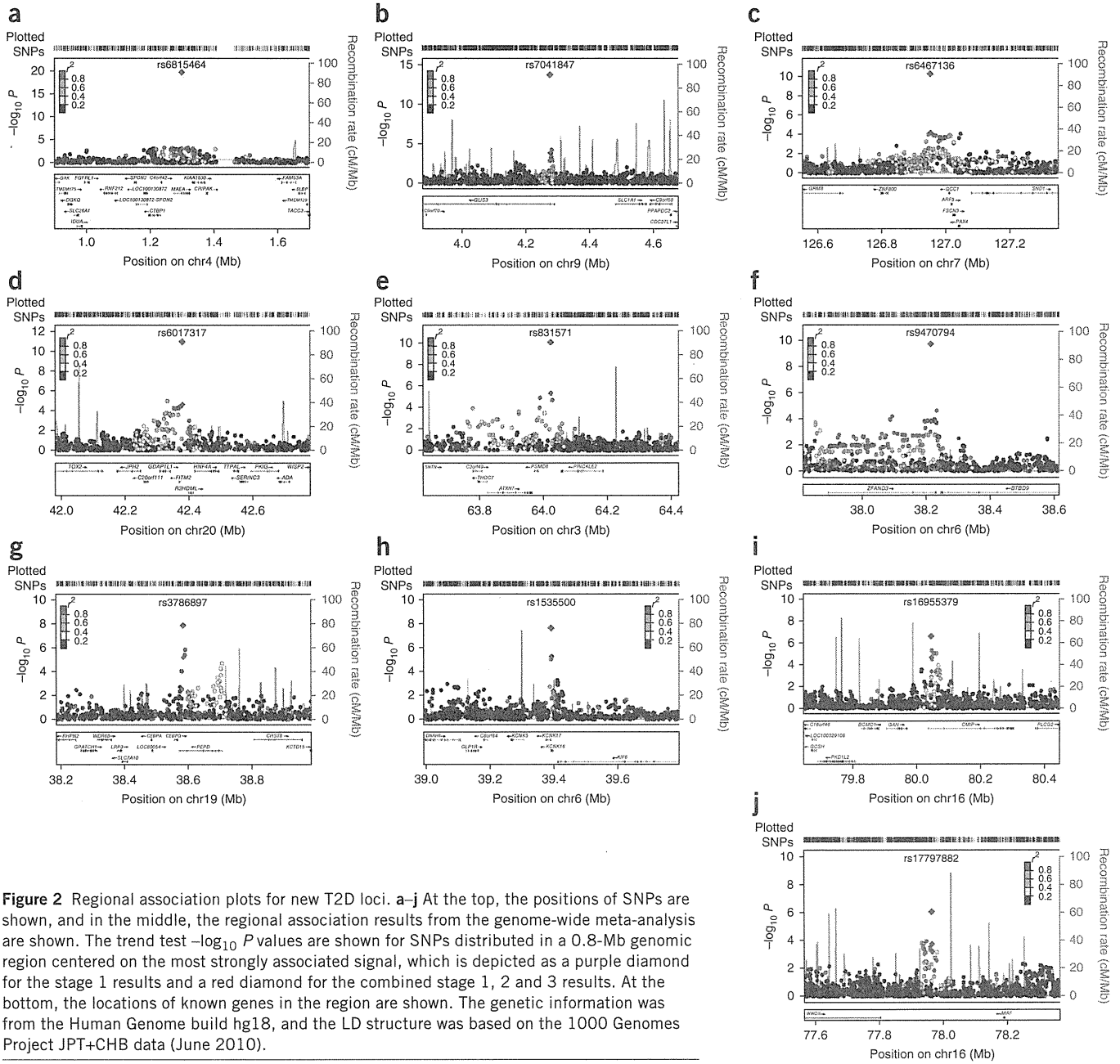

Figure 2 Regional association plots for new T2D loci. **a–j** At the top, the positions of SNPs are shown, and in the middle, the regional association results from the genome-wide meta-analysis are shown. The trend test $-\log_{10} P$ values are shown for SNPs distributed in a 0.8-Mb genomic region centered on the most strongly associated signal, which is depicted as a purple diamond for the stage 1 results and a red diamond for the combined stage 1, 2 and 3 results. At the bottom, the locations of known genes in the region are shown. The genetic information was from the Human Genome build hg18, and the LD structure was based on the 1000 Genomes Project JPT+CHB data (June 2010).

rs11634397 near *ZFAND6* seems to be specific to European populations (**Supplementary Table 3**). We observed a substantial difference in the risk allele frequencies of both loci between the two continental (Asian and European) populations (rs9470794: risk allele frequency (RAF) = 0.32 for the Asian CHB/JPT HapMap population compared to RAF = 0.12 for the European CEU HapMap population; rs11634397: RAF = 0.07 for CHB/JPT compared to RAF = 0.64 for CEU). Although these loci are related to T2D differently in the two populations (the *ZFAND3* locus is specific to Asians, whereas the *ZFAND6* locus to Europeans), these results lead to speculation that the broader A20 domain-containing zinc finger protein family has a role in the etiology of T2D. Additional population-specific T2D loci were also suggested by our analysis, for example, *WWOX* (rs17797882) (**Supplementary Table 5**) in east Asians and *ZBED3* (rs4457053) (**Supplementary Table 3**) in Europeans. Despite the lack of clear physiological evidence on T2D pathogenesis, these findings may provide clues to help understand T2D phenotypes characteristic of each population, for example, the high rates of diabetes seen at lower average BMIs in east Asians.

URLs. IMPUTE, http://mathgen.stats.ox.ac.uk/impute/impute.html; MACH, http://www.sph.umich.edu/csg/abecasis/MACH/; BEAGLE, http://faculty.washington.edu/browning/beagle/beagle.html; METAL, http://www.sph.umich.edu/csg/abecasis/Metal; WGAViewer, http://compute1.lsrc.duke.edu/softwares/WGAViewer/; SNAP, http://www.broadinstitute.org/mpg/snap/; LocusZoom, http://csg.sph.umich.edu/locuszoom/; GenABEL, http://www.genabel.org/; ProbABEL, http://www.genabel.org/packages/ProbABEL.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

The study was supervised by E.S.T., B.-G.H., N.K., Y.S.C., Y.Y.T., W.Z., Q.C., X.O.S., Y.-T.C., J.-Y.W., L.S.A., K.L.M., T.K., C.H., W.J., L.-M.C., Y.M.C., K.S.P., J.-Y.L. and J.C.N.C. The experiments were conceived of and designed by Y.S.C., E.S.T., N.K., D.P.-K.N., J.J.-M.L., M.S., T.Y.W., Y.Y.T., W.Z., F.B.H., X.O.S., C.-H.C., F.-J.T., Y.-T.C., J.-Y.W., L.S.A., K.L.M., S.M., C.H., L.-M.C., K.S.P., M.J.G., M.I.M. and R.C.W.M. The experiments were performed by J.L., M.S., J.J.L., J.-Y.W., S.M., R.Z., K.Y., Y.-C.C., T.-J.C., L.-M.C. and S.H.K. Statistical analyses was performed by M.J.G., X.S., Y.J.K., R.T.H.O., W.T.T., Y.Y.T., F.T., J.L., C.-H.C., L.-C.C., Y.W., Y.L., K.H., C.H., Y.-C.C., S.H.K., A.P.M. and R.C.W.M. The data were analyzed by M.J.G., X.S., Y.J.K., R.T.H.O., W.T.T., Y.Y.T., J.L., C.-H.C., L.-C.C., Y.W., N.R.L., Y.L., L.S.A., K.L.M., T.Y., C.H., Y.-C.C., S.H.K., Y.S.C., S.K., Å.K.H. and R.C.W.M. The reagents, materials and analysis tools were contributed by E.S.T., B.-G.H., N.K., D.P.-K.N., J.J.-M.L., J.L., M.S., T.A., T.Y.W., E.N., M.Y., J.N., J.J.L., W.Z., Q.C., Y.G., W.L., F.B.H., X.O.S., F.-J.T., Y.-T.C., J.-Y.W., N.R.L., Y.L., K.O., H.I., R.T., C.W., Y.B., T.-J.C., L.-M.C., K.S.P., H.-L.K., N.H.C., J.-Y.L., W.Y.S. and J.C.N.C. The manuscript was written by Y.S.C., M.S. and E.S.T. All authors reviewed the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Study subjects. Stage 1 subjects were drawn from eight T2D GWAS participating in the AGEN consortium, which was organized to enable genetic studies on diverse complex traits in 2010. These eight studies included 6,952 cases with T2D and 11,865 controls from the Korea Association Resource Study (KARE), the Singapore Diabetes Cohort Study (SDCS), the Singapore Prospective Study Program (SP2), the Singapore Malay Eye Study (SiMES), the Japan Cardiometabolic Genome Epidemiology Network (CAGE), the Shanghai Diabetes Genetic Study (SDGS), the Taiwan T2D Study (TDS) and the Cebu Longitudinal Health and Nutritional Survey (CLHNS). Subjects in stage 2 included 5,843 cases with T2D and 4,574 controls from three independent GWAS, the BioBank Japan Study (BBJ), the Health2 T2D Study (H2T2DS) and the Shanghai Jiao Tong University Diabetes Study (SJTUDS), for *in silico* replication analysis. Stage 3 included up to 12,284 cases with T2D and 13,172 controls from five different studies, the Japan Cardiometabolic Genome Epidemiology Network (CAGE), the Shanghai Diabetes Study I/II (SDS I/II), the Chinese University of Hong Kong Diabetes Study (CUHKDS), the National Taiwan University Hospital Diabetes Study (NTUHDS) and the Seoul National University Hospital Diabetes Study (SNUHDS), for *de novo* replication analysis. The study design and T2D diagnosis criteria used in each study included in stages 1, 2, and 3 are described in **Supplementary Table 1** and the **Supplementary Note**. Each study obtained approval from the appropriate institutional review boards of each participating institution, and written informed consent was obtained from all participants. The three-stage design of the overall study is depicted in **Supplementary Figure 1**.

Genotyping and imputation. Subjects for the stage 1 and 2 analyses were genotyped with high-density SNP typing platforms that covered the entire human genome. In most of the studies, only unrelated samples with missing genotype call rates below 5% were included for subsequent GWAS analyses. For the genome-wide association meta-analysis, each study participating in stages 1 and 2 performed SNP imputation. IMPUTE, MACH or BEAGLE (see URLs) were used, together with haplotype reference panels from the JPT and CHB samples that are available in the HapMap database (JPT+CHB+CEU and/or YRI, in some studies) on the basis of HapMap build 36 (release 21, 22, 23a or 24). Only imputed SNPs with high genotype information content (proper info > 0.5 for IMPUTE and Rsq > 0.3 for MACH and BEAGLE) were used for the association analysis. Genotyping for the stage 3 analysis was carried out using TaqMan, Sequenom MassARRAY or the Beckman SNP Stream method. All SNPs included in stage 3 had a genotype success rate of >98% (**Supplementary Table 2**).

Statistical analyses, analysis tools and SNP prioritization for stages 2 and 3. Associations between SNPs and T2D were tested by logistic regression with an additive model (1 degree of freedom) after adjustment for sex. Other adjustments were permitted according to the situations in the individual studies. The meta-analysis was performed using an inverse-variance method assuming fixed effects, with a Cochran's Q test to assess between-study heterogeneity. METAL software (see URLs) was used for all meta-analyses. A plot of the negative log of the association results from the stage 1 meta-analysis, by chromosome, was generated using WGAViewer software (see URLs).The quantile-quantile plot was constructed by plotting the distribution of observed P values for the given SNPs against the theoretical distribution of the expected P values for T2D[34]. The genomic control inflation factor, λ, was calculated by dividing the median χ^2 statistics by 0.456 (ref. 35) for individual GWAS, as well as for the stage 1 GWAS meta-analysis. We did not correct for genomic control in the stage 1 analyses because the inflation was modest, suggesting that population structure is unlikely to cause substantial inflation of the stage 1 results (**Supplementary Table 2**). The selection criteria for the lead SNPs to take forward to stage 2 *in silico* replication analysis were as follows: (i) stage 1 meta-analysis $P < 5 \times 10^{-4}$ (based on the divergence between the observed and expected P values on the quantile-quantile plot; **Supplementary Fig. 2**); (ii) heterogeneity $P > 0.01$; and (iii) at least seven studies having been included in the stage 1 meta-analysis (**Supplementary Table 4**). After removing known variants associated with T2D, proxies for each lead SNP ($r^2 > 0.8$) were selected using the SNAP software (see URLs). The replication genotyping for stage 3 was performed for the new SNPs having a stage 2 combined $P < 10^{-5}$. Regional association results from genome-wide meta-analysis were plotted using LocusZoom software (see URLs) for SNPs reaching genome-wide significance from the combined meta-analysis of stages 1, 2 and 3.

Principal components analysis. A list of 76,534 common SNPs across the Illumina 550, 610 and 1M and Affymetrix 5.0 and 6.0 arrays were first selected. This set of SNPs in the Asian (CHB+JPT) HapMap II samples was then trained to generate a list of 44,524 SNPs having pairwise LD < 0.3 in a sliding window of 50 SNPs. Individuals from each component study and from HapMap II were plotted based on the first two eigenvectors produced by the principal components analysis.

eQTL analysis. Gene expression information from 776 adipose tissues, 667 skin tissues and 777 lymphoblastoid cell lines was obtained from the MuTHER consortium[36]. The eQTL data for eight of the ten T2D loci identified in this study were available in the MuTHER dataset. Most of those loci passed the filtering criteria, such as MAF > 5% and INFO > 0.8, except for rs16955379, which has MAF = 1.5% in the MuTHER data set. Two of the ten loci that were used in the eQTL analysis, rs6815464 (on chromosome 4) and rs17797882 (on chromosome 16), are not included in the MuTHER data set. Association between each SNP with a significant association to T2D and the normalized mRNA expression values of genes within 1 Mb of the lead SNP were performed with the GenABEL and ProbABEL package (see URLs) using the polygenic linear model incorporating a kinship matrix in GenABEL followed by the ProbABEL mmscore test with imputed genotypes. A multiple-testing correction was applied to the *cis* association results. P value thresholds of $P = 5.06 \times 10^{-5}$ in adipose tissue, $P = 3.81 \times 10^{-5}$ in skin and $P = 7.80 \times 10^{-5}$ in lymphoblastoid cell lines correspond to an estimated genome-wide false discovery rate of 1%.

Gene relationships among implicated loci (GRAIL) analysis. A GRAIL analysis was performed as described previously[31,33]. A total of 38 genes within T2D-associated regions were selected for the analysis. Among these genes, 28 were from the previously implicated set (**Supplementary Table 3**), and the other 10 genes were newly implicated in this study (**Table 1**). PubMed abstracts published after December 2006 were omitted from the analysis to reduce confounding by results from T2D GWAS.

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Genetics and pathogenesis of type 1 diabetes: prospects for prevention and intervention

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ABSTRACT

Type 1 diabetes is etiologically a multifactorial disease caused by a complex interaction of genetic and environmental factors, with the former consisting of multiple susceptibility genes. Identification of genes conferring susceptibility to type 1 diabetes would clarify etiological pathways in the development and progression of type 1 diabetes, leading to the establishment of effective methods for prevention and intervention of the disease. Among multiple susceptibility genes, *HLA* and *INS* are particularly important because of their contribution to tissue specificity in the autoimmune process. *DRB1*04:05-DQB1*04:01* is associated with autoimmune type 1 diabetes, idiopathic fulminant type 1 diabetes and anti-islet autoimmunity in autoimmune thyroid diseases, suggesting that this haplotype is associated with beta-cell specificity in autoimmune diseases. Genes involved in the expression of insulin in the thymus contribute to beta-cell-specific autoimmune mechanisms in type 1 diabetes. These genes and pathways are important targets for tissue-specific prevention and intervention of type 1 diabetes. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00176.x, 2011)

KEY WORDS: Autoimmune disease, Genetics, Type 1 diabetes

INTRODUCTION

Type 1 diabetes is caused by destruction of insulin-producing beta-cells of the pancreas in genetically susceptible individuals. Etiologically, type 1 diabetes is classified into two major subtypes, autoimmune (type 1A) and idiopathic (type 1B). The etiologic factors and pathogenesis of idiopathic type 1 diabetes are still unknown, but recent studies suggested that fulminant type 1 diabetes may belong to this subtype[1,2]. Type 1A diabetes is an organ-specific autoimmune disease in which beta-cells of the pancreas are the target organ of the autoimmune attack.

Type 1 diabetes is a multifactorial disease caused by a complex interaction of genetic and environmental factors, with the former consisting of multiple susceptibility genes. Identification of genes conferring susceptibility to type 1 diabetes would clarify the etiological pathways in the development and progression of type 1 diabetes, leading to the establishment of effective methods for prevention and intervention of the disease. In this review, clinical problems in the treatment of type 1 diabetes are summarized in order to help understand the reason why identification of genes conferring susceptibility to type 1 diabetes is necessary, and then the current status of the molecular genetics of type 1 diabetes is reviewed with special emphasis on genes that contribute to tissue specificity of autoimmune mechanisms.

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WHY GENES?

Among patients with type 1 diabetes, heterogeneity of residual beta-cell function is observed. Some patients completely lack endogenous insulin secretion, while others have preservation of minimal insulin secretory capacity. Complete lack of endogenous insulin secretion in type 1 diabetes is associated with unstable glycemic control, so-called brittle diabetes, as evidenced by our previous studies showing an inverse correlation between unstable glycemic control and minimal residual beta-cell function in type 1 diabetes[3]. These data have recently been confirmed in fulminant diabetes[4]. This can be explained by the buffering action of endogenous insulin, whose secretion, even in a small amount, is automatically adjusted to the body's need on a minute-to-minute basis. Excess exogenous insulin can be adjusted by a decrease in endogenous insulin, whereas deficiency of insulin can be adjusted by a small increase in endogenous insulin. Type 1 diabetic patients with no residual beta-cell function lack this buffering action of endogenous insulin, and therefore have difficulty maintaining stable glycemic control, even with continuous subcutaneous insulin infusion (CSII). At the moment, pre-programmable CSII may be the only way to achieve glycemic control in such patients. Figure 1 shows the basal insulin infusion rate of pre-programmable CSII to achieve stable glycemic control in five patients with type 1 diabetes with complete lack of endogenous insulin. To achieve stable glycemic control, very dynamic adjustment of basal insulin infusion was required, with a decrease in infusion rate to avoid nocturnal hypoglycemia and an increase in infusion rate to overcome the dawn phenomenon. This in turn suggests that type 1 diabetic patients, particularly those with complete lack of endogenous